

单种属弥勒苣苔属系统位置研究：基于分子和细胞学数据*

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摘要：弥勒苣苔属是苦苣苔科的单种属，仅分布于中国西南部。为探讨弥勒苣苔在苦苣苔亚科中的系统位置，我们选择了苦苣苔亚科 116 个类群，外类群为苦苣苔亚科以外的 7 个物种。用最大简约法（MP）和贝叶斯分析（BI），对以上类群的核基因 ITS 以及两个叶绿体基因 *trnL-F*、*atpB-rbcL* 数据进行了独立和联合分析。在三个片段联合分析的结果中，弥勒苣苔与马铃苣苔属、后蕊苣苔属、金盏苣苔属、直瓣苣苔属以及川鄂粗筒苣苔构成一个强烈支持的分枝。MP 树中，此分枝为并系，而在 BI 分析中，弥勒苣苔与川鄂粗筒苣苔、直瓣苣苔属互为姐妹类群。同时，第一次报道了弥勒苣苔的染色体数目（ $2n=34$ ）。根据前人报道，马铃苣苔属、后蕊苣苔属、粗筒苣苔属和直瓣苣苔属的染色体数目同为 $2n=34$ ，这进一步支持我们的分子系统发育分析。

关键词：*atpB-rbcL*；苦苣苔科；ITS；弥勒苣苔属；系统学；*trnL-F*

中图分类号：Q 942, Q 949

文献标识码：A

文章编号：2095-0845(2011)05-465-12

The Systematic Placement of the Monotypic Genus *Paraisometrum* (Gesneriaceae) Based on Molecular and Cytological Data*

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Abstract: *Paraisometrum*, is a monotypic genus endemic to Southwest China, with an uncertain systematic position. To resolve the phylogenetic relationships of *Paraisometrum* within subfamily Cyrtandroideae, we performed phylogenetic analyses of 116 taxa placed in the subfamily Cyrtandroideae and 7 outgroup species selected from other Gesneriaceae subfamilies. The independent and combined analyses were performed on three DNA datasets: the nuclear internal transcribed spacer regions (ITS) and two chloroplast regions (*trnL-F*, *atpB-rbcL*). Maximum Parsimony (MP) and Bayesian Inference (BI) were used for tree construction. The combined three gene dataset strongly sup-

* Foundation items: The Chinese Academy of Sciences (KSCX2-YW-Z-0925 & O92441112F), the Ministry of Education of China through its 985 and 111 projects (MUC 98503-001006 & B08044), the National Science Foundation of China (31070288), the Japan Society for the Promotion of Science (JSPS/AP/109080), and the Ministry of Science and Technology of China (2008FY110400-2-2)

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Received date: 2011-05-10, Accepted date: 2011-06-16

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port *Paraisometrum* in a clade containing *Oreocharis*, *Opithandra*, *Isometrum*, *Ancylostemon*, and *Briggsia rosthomii*. In the MP tree this lineage is an unresolved polytomy, but BI resolves *Paraisometrum* as sister to *B. rosthomii* and the *Ancylostemon* clade. We did the first chromosome counts of *Paraisometrum* ($2n=34$). The diploid number 34 has also been reported for *Oreocharis*, *Opithandra*, *Briggsia* and *Ancylostemon*, which lends further support to the placement of *Paraisometrum* in this lineage.

Key words: *atpB-rbcL*; Gesneriaceae; ITS; *Paraisometrum*; Phylogeny; *trnL-F*

Paraisometrum W. T. Wang is a monotypic genus, containing *P. mileense* W. T. Wang, which is endemic to Southwest China (Weitzman *et al.*, 1997) in the Yunnan Province (Shui, 2007a, b) and Guangxi Region (Xu *et al.*, 2009). The holotype of this plant was collected by French missionary Ducloux F. in Mi-le County, Yunnan Province in 1906 and remained unnamed and stored in Paris for nearly 100 years. In 1997, when Weitzman *et al.* wrote an article on Gesneriaceae in the corpus for Flora of China, Prof. W. T. Wang checked Ducloux's specimens in Paris collected from Mi-le County and concluded that it represented a new genus in Gesneriaceae and named it *Paraisometrum mileense* (Weitzman *et al.*, 1997). It was believed extinct until 2006. In 2006, the species was rediscovered in the Shilin County, Yunnan Province by Dr. Yuming Shui, a botanist from the Kunming Institute of Botany, Chinese Academy of Sciences (Shui, 2007a, b). According to the IUCN Species Programme, *Paraisometrum mileense* can be considered an endangered species because of its narrow geographic range and small population size.

The Chinese Gesneriaceae, based on morphological characteristics, have been divided into six tribes: Ramondeae, Didymocarpeae, Trichosporeae, Cyrtandreae, Epithemateae and Titanotricheae (Wang *et al.*, 1992). Wang *et al.* (1998) placed *P. mileense* in the Didymocarpeae, and stated that it is most similar to the genus *Isometrum* Craib because they both have four fertile stamens and a funnelform tube, longer than the lobes. More recently, molecular analyses of subfamily Didymocarpoideae have been conducted by Mayer *et al.* (2003) and Möller *et al.* (2009) based on chloroplast DNA (cpDNA)

or combined ITS and cpDNA sequences, respectively. Neither study included samples of *P. mileense*, which left its phylogenetic position unresolved. The rediscovery of a population of *P. mileense* have made it possible to determine the phylogenetic position of this species to test the morphological hypothesis of Wang *et al.* (1998).

To investigate the systematic position of *Paraisometrum*, we used DNA sequences for the nuclear internal transcribed spacer regions (ITS), cpDNA *trnL-F* intron-spacer region (*trnL-F*) and *atpB-rbcL* spacer (*atpB-rbcL*). In addition, we report the chromosome numbers of *Paraisometrum* for the first time and discuss characters among closely related genera.

1 Materials and methods

1.1 Taxon sampling

To produce a reliable phylogeny of the subfamily Cyrtandroideae the ingroup comprised 116 taxa, representing 115 species in 46 genera and included 4 members of Gesnerioid and 3 members of Coronantheroid as outgroups (Appendix). These outgroup taxa were chosen based on results of recent studies of Gesneriaceae (Zimmer *et al.*, 2002; Perret *et al.*, 2003; Möller *et al.*, 2009). Sequences used in this study were from GenBank and supplemented with 17 new sequences (5 individuals of *trnL-F*, 6 *atpB-rbcL*, 6 ITS). For the ingroup we followed the taxonomic classification of Weber (2004). 13 species (5 genera) belonged to Epithematoid Gesneriaceae and 103 species (42 genera) came from Didymocarpoid Gesneriaceae (Appendix).

1.2 Molecular methods

Materials for DNA extraction were from various

forms and diverse sources, including fresh leaves from research collections, silica-dried leaves from our field collections. Genomic DNA was isolated with a modified CTAB protocol (Doyle and Doyle, 1987).

Amplifications of ITS were carried out following the PCR profile described by Möller and Cronk (1997), using forward primer 'ITS 5P' and reverse primer 'ITS 8P'. The *trnL-F* intron-spacer region (*trnL-F*) and *atpB-rbcL* spacer (*atpB-rbcL*) were amplified following Mayer *et al.* (2003) using primers 'c' and 'f' for *trnL-F* (Taberlet *et al.*, 1991) and primers 'JF31' and 'JF5' for *atpB-rbcL* (Samuel *et al.*, 1997). PCR was performed using a DNAEngine[®] peltier thermal cycler (Bio-RAD, Inc.). The PCR products were purified using a multifunction DNA purification KIT (BioTeke Inc.). The purified PCR products were cycle-sequenced using an ABI Prism BigDye Terminator Cycle Kit following standard kit protocols (Perkin-Elmer Applied Biosystems) and run on a ABI-PRISM3730 sequencer. Forward and reverse sequences were assembled using SeqMan of DNASTar (DNASTAR, 1999), visually aligned with ClustalX v. 1.83 (Thompson *et al.*, 1998) followed by manual correction in BioEdit (Hall, 1999). Sequence alignments and PAUP/Nexus-formatted files for individual regions and combined analyses are available from the authors upon request.

1.3 Phylogenetic analysis

Phylogenetic analyses were performed using Maximum Parsimony (MP) and Bayesian Inference (BI) using the protocols of Rannala and Yang (1996) on the individual ITS, *atpB-rbcL*, *trnL-F*, combined cpDNA and combined three gene datasets respectively. Maximum Parsimony analysis was performed with PAUP* version 4.0b10 (Swofford, 2002). Gaps were treated as missing data, and characters were assumed to be unordered. Heuristic searches were performed employing 1 000 random replicates, holding one tree at each step during stepwise addition, using tree-bisection-reconnection (TBR) branch-swapping algo-

rithm, MulTrees in effect, and steepest descent off. Support for the nodes resolved in the strict consensus of the MP trees was calculated by bootstrap analyses (Felsenstein, 1985) with 1 000 heuristic-search replicates as described above. Bayesian analysis was performed using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2003) for all the datasets. The most appropriate molecular model for each dataset was determined with jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008). The model GTR+I+G was selected. The Markov chain Monte Carlo (MCMC) algorithm was run for 10 000 000 generations with one cold and three heated chains in two independent parallel analyses. Trees were sampled every 100 generations from the chain. The first 20% trees were discarded as burn-in (generations prior to stationary of likelihood values).

1.4 Cytological protocols

Root tips of *Paraisometrum mileense* were pretreated in $0.002 \text{ mol} \cdot \text{L}^{-1}$ 8-hydroxyquinoline for 4 to 5 h at room temperature, and then fixed in Carnoy's I (3 ethanol : 1 glacial acetic acid) for 30 min at 5°C . After hydrolysis for 1 min in $1 \text{ mol} \cdot \text{L}^{-1}$ HCl at 60°C , followed by washing through several changes of distilled water, the root tips were dyed in carbol fuchsin and then squashed. The chromosomes were examined using Axioskop 40 made by ZEISS and photographed using JVC TK-C1480BEC color video camera.

2 Results

2.1 Phylogenetic analysis

The main clades found in the parsimony analyses of the individual ITS, *trnL-F*, *atpB-rbcL* and combined cpDNA datasets (not shown) were broadly congruent with, although less well resolved than, the consensus trees from the combined three gene dataset. This reflects the fewer of informative characters in the separate analyses. The results of the combined three gene analyses are presented here.

Of the aligned 2990 characters (ITS: 791 characters, *trnL-F*: 1189 characters, *atpB-rbcL*: 1010

characters) analyzed, 1100 were constant, 549 uninformative, and 1341 parsimony informative. Maximum parsimony analysis resulted in 4 most parsimonious trees of 7058 steps, with a CI of 0.44, a RI of 0.68, and a RC of 0.30. A strict consensus tree of the combined dataset resolved 92 nodes with bootstrap support (BS) $\geq 50\%$ (Fig. 1). The phylogram from the Bayesian analysis of the combined dataset resolved 113 nodes, 95 of which had posterior probabilities (PP) ≥ 0.90 (Fig. 2). Support for deep nodes was weak, but the support for the individual major clades was strong.

The MP strict consensus tree had a similar tree topology to that of Bayesian consensus tree. Subfamily Cyrtandroideae is monophyletic and sister to subfamily Gesnerioideae (BS = 97%; PP = 100%). The tribe Epithemateae is sister to the remaining old world Gesneriaceae with strong support (BS = 100%; PP = 100%). Genus *Corallodiscus* form a clade (BS=87%; PP=100%) and is sister to the remaining taxa (BS=87%; PP=100%). Basal Asiatic genera, *Rhynchochelys*, *Platystemma*, and European genera, *Jancaea*, *Ramonda*, *Haberlea*, constitute a clade (BS=93%), which is separated from the African and remaining Asiatic genera with moderate support (BS=87%; PP=100%). Among the remaining taxa, *Briggsia*, *Chirita*, and *Streptocarpus* are not monophyletic. *Paraisometrum* is in a well supported clade (BS = 100%, PP = 100%) with *Briggsia rosthornii*, *Isometrum lungshengense*, *Opithandra primuloides*, *Oreocharis auricula* and three species of *Ancylostemon*. The three species of *Ancylostemon* form a monophyletic group with strong support (BS = 100%; PP = 100%). The BI tree provides more resolution in the *Paraisometrum* clade, *Paraisometrum* is sister to *Ancylostemon* and *Briggsia rosthornii* (PP=99%) in a polytomy with the other three genera of the lineage while the MP tree does not resolve any of the relationships among the genera in the clade.

2.2 Cytology

Paraisometrum mileense has a diploid number of

$2n=34$, and the chromosome size is small, $<2\ \mu\text{m}$ (Fig. 3).

3 Discussion

3.1 Phylogenetic relationships

In our study, Old World Gesneriaceae can be clearly divided into two tribal groups that are congruent with previous studies (Smith *et al.*, 1997; Wang and Li, 2002; Mayer *et al.*, 2003; Möller *et al.*, 2009; Wang *et al.*, 2010). Weber (2004) separated subfamily Cyrtandroideae into the small tribe Epithemateae (Epithematoid Gesneriaceae) and the large tribe Didymocarpeae in which the members of Cyrtandreae and Trichosporeae were polyphyletically nested at various places (Didymocarpoid Gesneriaceae). On the basis of morphological and geographical features, the Didymocarpoid Gesneriaceae can be divided into following groups: Basal Asiatic and European genera, African genera, Advanced Asiatic genera (including genera with twisted capsular fruits, straight capsular fruits, or indehiscent fruits). The Asiatic and European, and African groups are well defined and supported according to the phylogenetic result made by Möller *et al.* (2009). In their study, the genus *Briggsia* is also not monophyletic, and *Oreocharis*, one clade of *Briggsia* and *Ancylostemon* compose a clade. Our results show that *Oreocharis*, *Opithandra*, *Isometrum*, *Ancylostemon*, *Briggsia rosthornii* and *Paraisometrum* constitute a clade in the Advanced Asiatic group, and suggest *Paraisometrum* may be mostly related to *Ancylostemon* and *Briggsia rosthornii* than other genera.

3.2 Morphology

The 5 genera in the clade with *Paraisometrum* have some shared floral characters, supporting their relationship. These genera have campanulate calyx, ad basin 5-sectus, bilabiate corollas, corolla lobes much shorter than the corolla tube, and 4 fertile stamens, stigma number varies, *Isometrum*, *Briggsia*, and *Ancylostemon* have 2 stigmas, whereas *Opithandra* and *Oreocharis* can have either 1 or 2 stigmas, and *Paraisometrum* always has a single stigma (Wang *et al.*, 1998).

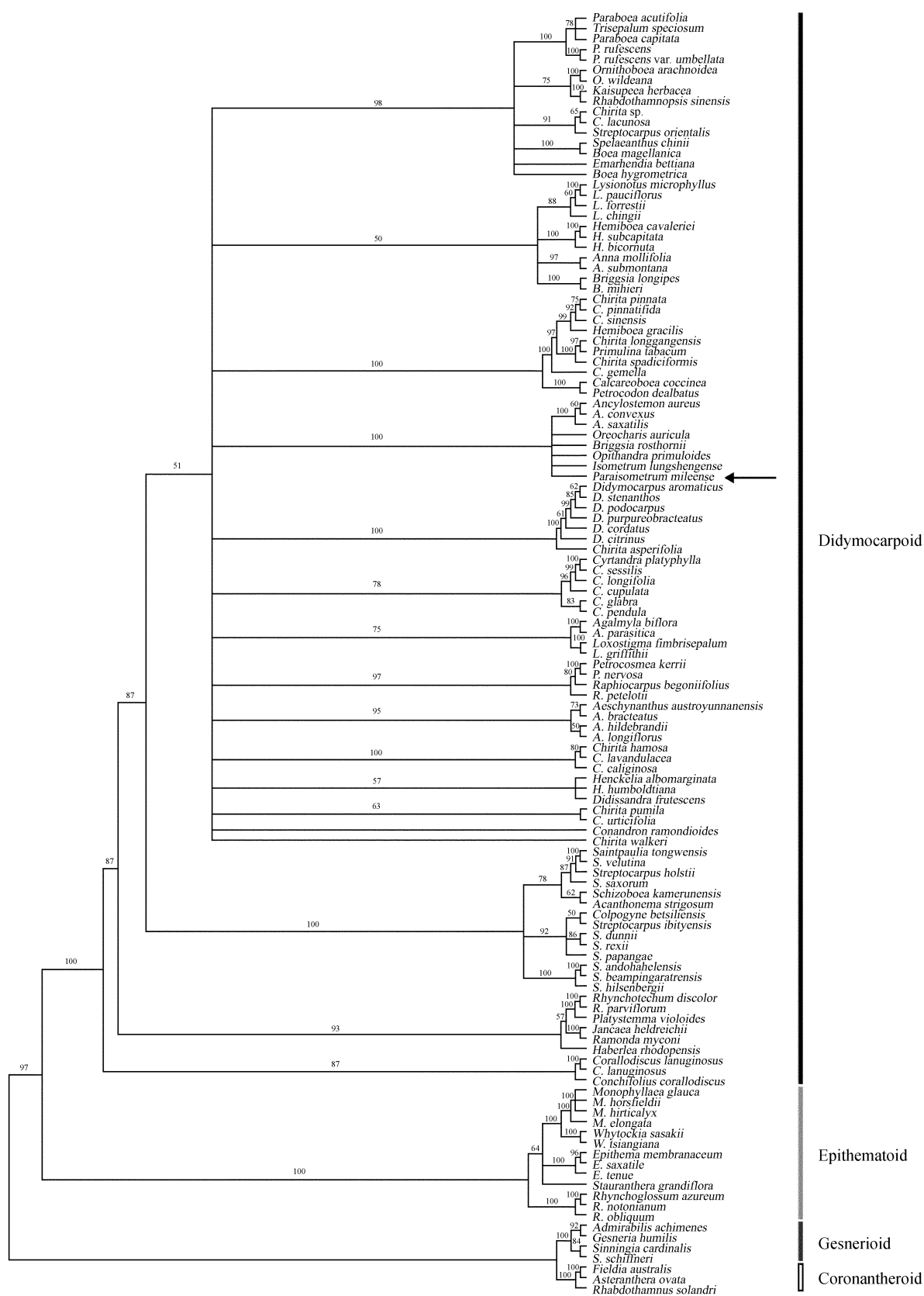
Fig. 1 Strict consensus tree of 4 most parsimonious trees based on the combined ITS, *trnL-F* and *atpB-rbcL* data.Numbers above branches are bootstrap support values $\geq 50\%$. The tribes are labeled with bars



Fig. 2 Bayesian phylogram based on the combined ITS, *trnL-F* and *atpB-rbcL* data. Numbers above branches are posterior probability values (only values ≥ 0.90 are shown). The tribes are labeled with bars

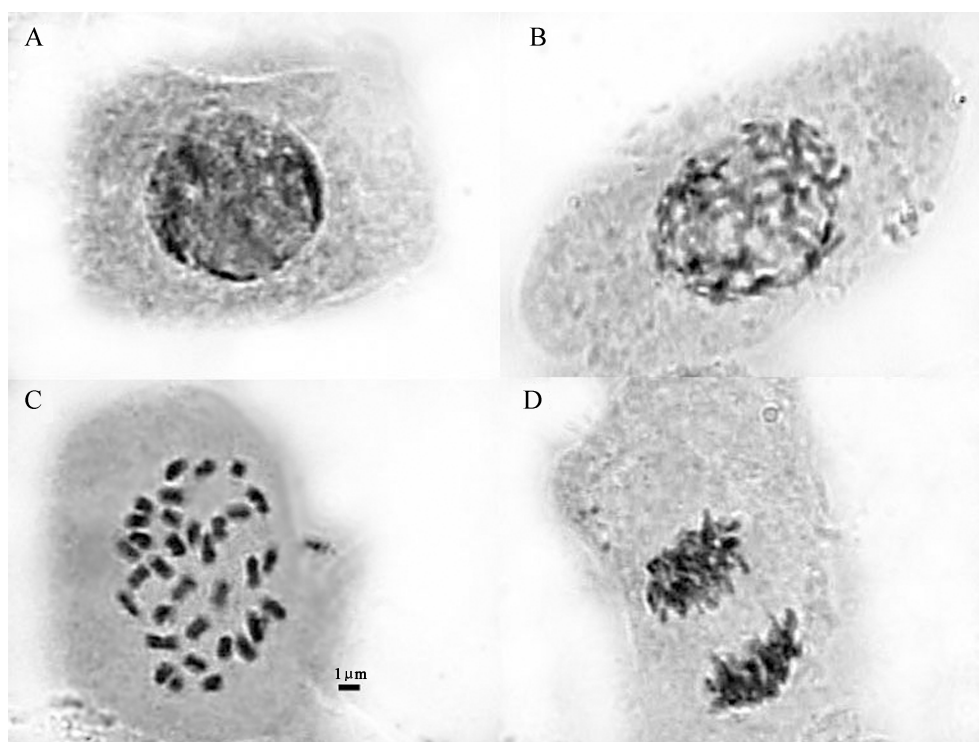


Fig. 3 Karyomorphology of *Paraisometrum mileense*. A. Interphase nucleus ($\times 1000$); B. Mitotic prophase ($\times 1000$); C. Mitotic metaphase ($\times 1000$); D. Mitotic anaphase ($\times 1000$). The bar is 1 μm long

Burt (1962) and Pan (1986) held the opinion that based on their observations that *Isometrum* Craib and *Ancylostemon* Craib were closely related, which our results support. Wang *et al.* (1998) hypothesized that *Paraisometrum* was sister to *Isometrum*. Our analysis confirmed that these two genera are closely related in the same lineage, but *Paraisometrum* likely has closer affinities to *Ancylostemon* and *Briggsia rosthornii*. Different from other 3 genera, their abaxial lip is longer than adaxial lip.

3.3 Distribution

Briggsia rosthornii is distributed in Sichuan, Guizhou, and Hubei provinces of Southwest and South-Central China, *Opithandra* is scattered throughout Western China, except for *O. primuloides*, a species restricted to Japan. The distributions of *Oreocharis* and *Ancylostemon* range from Southwestern to Central and Southeastern China (Li, 1996; Wang *et al.*, 1998; Li and Wang, 2004). *Isometrum* is endemic to China, and centered in Sichuan, where 82% of the species in the genus occur (Pan, 1986).

3.4 Cytology

Chromosome ploidy levels and base numbers can provide insight into plant evolution, because sister groups often share chromosome numbers or base number (Turner *et al.*, 1961; Ehrendorfer *et al.*, 1968; Walker, 1972; Raven, 1975; Grant, 1982). However, chromosome counts at the species-level across the family have been made for only about 18% of the taxa in the Gesneriaceae, and the number of genera without chromosome counts is even greater in the subfamily Cyrtandroideae than subfamily Gesnerioideae (Möller and Kiehn, 2004). In the Epithemateae, the chromosome counts have high variation, with the basic numbers in this group ranging from $n = 8 - 12$, (Ratter, 1975; Skog, 1984; Wang *et al.*, 1998). It is more complicated in the Didymocarpoideae Gesneriaceae, where basic numbers are $n = 4, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18$ (Ratter and Prentice, 1967; Ratter, 1975; Kiehn and Weber, 1998; Rashid *et al.*, 2001; Möller and Kiehn, 2004). In previous studies, the genera

Oreocharis, *Opithandra*, and *Ancylostemon* have the same chromosome number $2n=34$, *Briggsia* has the chromosome number $2n=34$ or $2n=68$ (Ratter, 1963; Ratter and Prentice, 1964; Wang and Gu, 1999; Zhou *et al.*, 2004). *Paraisometrum* also has a chromosome complement of $2n=34$, which is another character uniting members of this clade. The chromosome is too small to do karyotype analysis, or we can do further comparison of these species. Although the data are not yet available, we predict that *Isometrum* to have a karyotype of $2n=34$.

Our molecular analyses place the rare monotypic genus, *Paraisometrum*, among the Didymocarpoideae genera, subfamily Cyrtandroideae of Gesneriaceae. *Paraisometrum* belongs in a strongly supported clade with *Oreocharis*, *Opithandra*, *Isometrum*, *Ancylostemon*, and *Briggsia rosthornii* among and nested in an informal group the Advanced Asiatic group. Bayesian analyses support *Paraisometrum*, as sister to *Ancylostemon* and *Briggsia rosthornii*. Because *Briggsia rosthornii* is a single species (and not the type) in a polyphyletic genus further study and taxonomic revision will be required on this clade. So at this time the decision on whether *Briggsia rosthornii* should be considered another monotypic genus or whether it and/or *Paraisometrum mileense* should be combined into an existing genus remain unanswered.

Acknowledgement: We thank Prof. Lei Shi from the Botanical Garden of Institute of Botany, CAS, for providing some plant materials, Mr. Yu Guo for laboratory assistance, and Dr. Yanchun Li, Dr. Yuxiao Zhang, Dr. Wenbin Yu, Dr. Bo Long and Dr. Jiahui Chen for advice on data analysis.

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Appendix: GenBank accession numbers for the sequences used in the phylogenetic analysis

Taxon	Locality	Voucher	GenBank accession No.		
			<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
Outgroup taxon					
Gesnerioid					
<i>Achimenes admirabilis</i> Wiehler			AJ439982	AJ439827	AY182173
<i>Sinningia cardinalis</i> (Lehm.) H. E. Moore			AJ490931	AJ492318	—
<i>S. schiffneri</i> Fritsch			AJ439900	AJ439745	—
<i>Gesneria humilis</i> L.			AJ439976	AJ439821	AY047051
Coronanthroid					
<i>Asteranthera ovata</i> (Cav.) Hanst			FJ501371	FJ501427	EF445669
<i>Fiddia australis</i> Cunn.			AY423112	AY423130	EF445687
<i>Rhabdothamnux solandri</i> Cunn.			FJ501370	FJ501426	EF445700
Ingroup taxon					
Didymocarpoid					
<i>Acanthonema strigosum</i> Hook. f.			—	FJ501454	FJ501306
<i>Aeschynanthus austroyunnanensis</i> W. T. Wang			FJ501396	FJ501500	AF349218/AF349299
<i>A. bracteatus</i> Wall. ex DC.			—	FJ501501	AF349203/AF349284
<i>A. hildebrandtii</i> Hemsl.			—	AY047099	AY047040
<i>A. longiflorus</i> (Blume) DC.			AJ490920	AJ492307	FJ501333
<i>Agalmyla biflora</i> (Elmer) O. M. Hilliard & B. L. Burt			FJ501421	FJ501541	FJ501361
<i>A. parasitica</i> (Lam.) Kuntze			FJ501420	FJ501539	—
<i>Ancylostemon aureus</i> (Franch.) B. L. Burt			FJ501398	FJ501505	FJ501336
<i>A. convexus</i> Craib			—	FJ501506	FJ501337
<i>Anna mollifolia</i> (W.T.Wang) W.T.Wang & K.Y.Pan			—	FJ501543	AF055050/AF055051
<i>A. submontana</i> Pellegr.			FJ501422	FJ501542	FJ501362
<i>Boea hygrometrica</i> (Bunge) R. Br.			—	FJ501476	FJ501319
<i>B. magellanica</i> Lam.			—	FJ501478	FJ501321
<i>Briggsia longipes</i> (Hemsl. Ex Oliv.) Craib			FJ501423	FJ501545	AF055052/AF055053
<i>B. mihieri</i> Craib			—	FJ501544	FJ501363
<i>B. rosthornii</i> (Diels) B. L. Burt			FJ501425	FJ501547	FJ501365
<i>Calcareoboea coccinea</i> C. Y. Wu ex H. W. Li			FJ501406	FJ501516	FJ501365
<i>Chirita asperifolia</i> (Blume) B. L. Burt			FJ501419	FJ501538	FJ501359
<i>C. caliginosa</i> C. B. Clarke			FJ501391	FJ501488	FJ501325
<i>C. gemella</i> D. Wood			FJ501408	FJ501523	FJ501345
<i>C. hamosa</i> R. Br.			FJ501392	FJ501489	—
<i>C. lacunosa</i> (Hook f.) B. L. Burt			FJ501384	FJ501458	FJ501308
<i>C. lavandulacea</i> Stapf.			FJ501390	FJ501497	FJ501324
<i>C. longgangensis</i> W. T. Wang			AJ490903	AJ492290	FJ501347
<i>C. pinnata</i> W. T. Wang			—	FJ501526	FJ501349
<i>C. pinnatifida</i> (Hand. -Mazz.) B. L. Burt			—	FJ501527	FJ501350
<i>C. pumila</i> D. Don			FJ501393	FJ501491	FJ501327
* <i>Chirita</i> sp.	China, Yunnan Prov., Jiaozixueshan Mountain	HGWB-754 (KUN)	HQ327455	HQ327447	HQ327462
<i>C. sinensis</i> Lindl.			FJ501409	FJ501524	FJ501348
<i>C. spadiciformis</i> W. T. Wang			AJ490904	AJ492291	FJ501346
<i>C. urticifolia</i> Buch. -Ham. Ex D. Don			—	FJ501492	FJ501328
<i>C. walkei</i> Gardner			—	FJ501490	FJ501326
<i>Colpogyne betsiliensis</i> B. L. Burt			—	FJ501445	FJ501302
<i>Conandron ramondiioides</i> Sieb. & Zucc.			FJ501405	FJ501515	FJ501340
<i>Corallodiscus conchifolia</i> Batalin			FJ501375	FJ501433	—
<i>C. lanuginosus</i> (Wall. Ex R. Br) B. L. Burt			FJ501374	FJ501432	—

Continue

Taxon	Locality	Voucher	GenBank accession No.		
			<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
<i>C. lanuginosus</i> (Wall. Ex R. Br.) Burt	China, Yunnan, Nujiang	WZ2009CM03 (KUN)	HQ327458	HQ327450	HQ327465
<i>Cyrtandra cupulata</i> Ridl			FJ50141	FJ501532	AY818826/AY818861
<i>C. glabra</i> Banks ex Gaertn.			AY423119	AY423136	FJ501353
<i>C. longifolia</i> (Wawra) Hillebr. Ex C. B. Clarke			FJ501413	FJ501531	AY818846/AY818881
<i>C. pendula</i> Blume			FJ501412	FJ501530	FJ501354
<i>C. platyphylla</i> A. Gray			FJ501410	FJ591528	—
<i>C. sessilis</i> H. St. JohnKapua <i>et al.</i> s. n.			FJ501411	FJ501529	—
<i>Didissandra frutescens</i> (Jack) C. B. Clarke			U91313	FJ501521	—
<i>Didymocarpus aromaticus</i> Wall. ex D. Don			FJ501402	FJ501511	—
<i>D. citrinus</i> Ridul.			AJ490906	AJ492293	DQ912669
<i>D. cordatus</i> Wall. ex DC.			—	AJ492294	DQ912673
<i>D. podocarpus</i> C. B. Clarke			FJ501404	FJ501514	DQ912688
<i>D. purpureobracteatus</i> W. W. Sm			FJ501401	FJ501510	—
<i>D. stenanthos</i> C. B. Clarke			FJ501403	FJ501512	DQ912687
<i>Emarhendia bettiana</i> (M. R. Hend) Kiew			AJ490908	AJ492295	—
<i>Haberlea rhodopensis</i> Friv.			AJ490909	AJ492296	—
<i>Hemiboea bicornuta</i> (Hayata) Ohwi			FJ501416	FJ501534	FJ501356
<i>H. cavaleriei</i> H. Lev.			FJ501415	FJ501533	FJ501355
* <i>H. gracilis</i> Franch	China, Hunan, Zhangjiajie	WZ2010ZJJ01 (KUN)	HQ327453	HQ327445	HQ327461
<i>H. subcapitata</i> C. B. Clarke			FJ501417	FJ501535	FJ501357
<i>Henckelia albomarginata</i> (Hemsl.) A. Weber			AJ490910	AJ492297	—
<i>H. humboldtiana</i> (Gardner) A. Weber & B. L. Burt			FJ501389	FJ501485	—
<i>Isometrum lungshengense</i> (W. T. Wang) W. T. Wang & K. Y. Pan			—	GU350690	GU350659
<i>Jancaea heldreichii</i> Boiss.			FJ501378	FJ501439	—
<i>Kaisupeea herbacea</i> (C. B. Clarke) B. L. Burt			FJ501385	FJ501459	FJ501309
<i>Loxostigma fimbrisepalum</i> K. Y. Pan			FJ501399	FJ501507	—
<i>L. griffithii</i> (Wight) C. B. Clarke			FJ501400	FJ501508	FJ501338
<i>Lysionotus chingii</i> Chun ex W. T. Wang			—	FJ501498	FJ501332
<i>L. forrestii</i> W. W. Sm.			FJ501394	FJ501495	AF349152/AF349233
* <i>L. microphyllus</i> var. <i>microphyllus</i> W. T. Wang	China, Hunan, Zhangjiajie	WZ2010ZJJ03 (KUN)	HQ327456	—	HQ327463
<i>L. pauciflorus</i> Maxim.			FJ501395	FJ501497	FJ501331
<i>Opithandra primuloides</i> (Miq.) B. L. Burt			FJ501424	FJ501546	FJ501364
<i>Oreocharis auricular</i> (S. Moore) C. B. Clarke			—	FJ501482	FJ501323
<i>Ornithoboea arachnoidea</i> (Diels) Craib			FJ501387	FJ501461	FJ501312
<i>O. wildeana</i> Craib			—	FJ501462	FJ501313
<i>Paraboea acutifolia</i> (Ridl.) B. L. Burt			—	FJ501464	FJ501314
<i>P. capitata</i> Ridl. var. <i>capitata</i>			AJ490911	AJ492298	FJ501315
<i>P. rufescens</i> (Franch.) B. L. Burt			FJ501388	FJ501469	FJ501316
<i>P. rufescens</i> (Franch.) B. L. Burt var. <i>umbellata</i> (Drake) K. Y. Pan			—	FJ501470	FJ501317
* <i>Paraisometrum mileense</i> W. T. Wang	China, Yunnan, Shilin county	TY2009SL01 (KUN)	HQ327452	HQ327444	HQ327460
<i>Petrocodon dealbatus</i> Hance			FJ501418	FJ501538	FJ501358
<i>Petrocosmea kerrii</i> Craib			FJ501397	FJ501502	FJ501334

Continue

Taxon	Locality	Voucher	GenBank accession No.		
			<i>atpB-rbcL</i>	<i>trnL-F</i>	ITS1/ITS2
<i>P. nervosa</i> Craib			AJ4909012	AJ492299	FJ501335
<i>Platystemma violoides</i> Wall.			FJ501382	FJ501443	—
<i>Primulina tabacum</i> Hance			AJ490913	AJ492300	FJ501352
<i>Ramonda myconi</i> (L.) Rchb.			AJ490914	AJ492301	—
<i>Raphiocarpus begoniifolius</i> (H. Lev.) B. L. Burt			—	FJ501517	FJ501342
<i>R. petelotii</i> (Pellegr.) B. L. Burt			—	FJ501518	FJ501343
<i>Rhabdothamnopsis sinensis</i> Hemsl.			AJ490915	AJ492302	FJ501310
<i>Rhynchoetichum discolor</i> (Maxim.) B. L. Burt			FJ501376	FJ501436	—
<i>R. parviflorum</i> Blume			FJ501377	FJ501437	—
<i>Saintpaulia tongwensis</i> B. L. Burt			—	FJ501446	FJ501303
<i>S. velutina</i> B. L. Burt			AJ490916	AJ492303	FJ501304
<i>Schizoboea kamerunensis</i> K. Fritsch (B. L. Burt)			—	FJ501453	FJ501305
<i>Spelaeanthus chinii</i> Kiew			—	FJ501457	FJ501307
<i>Streptocarpus andohahelensis</i> Humbert			—	FJ501449	AF316903
<i>S. beampingararensis</i> Humbert var. <i>beampingararensis</i>			—	FJ501448	AF316905
<i>S. dunnii</i> Hook. f.			—	FJ501456	AF316951
<i>S. hilsenbergii</i> R. Br.			—	FJ501450	AF316907
<i>S. holstii</i> Engl.			AJ490917	AJ492304	AF316917
<i>S. ibityensis</i> Humbert			—	FJ501455	AF316926
<i>S. orientalis</i> Craib			—	FJ501444	AF316929
<i>S. papangae</i> Humbert			—	FJ501444	AF316929
<i>S. rexii</i> Lindl.			AJ490918	AJ492305	AF316979
<i>S. saxorum</i> Engl.			FJ501383	FJ501447	AF316914
<i>Trisepalum speciosum</i> (Ridl.) B. L. Burt			AJ490919	AJ492306	—
Epithematoid					
<i>Epithema membranaceum</i> (King) Kiew			AJ490887	AJ492274	—
<i>E. saxatile</i> Blume Weber & Anthonyamy			AJ490888	AJ492275	—
<i>E. taiwanense</i> S. S. Yin			AJ490889	AJ492276	—
<i>E. tenue</i> C. B. Clarke			AJ490890	AJ492277	—
<i>Monophyllaea elongate</i> B. L. Burt			AJ490892	AJ492279	—
<i>M. glauca</i> C. B. Clarke			AJ490893	AJ492280	—
<i>M. hircalyx</i> Franch.			AJ490894	AJ492269	—
<i>M. horfieldii</i> R. Br			U91315	AJ492269	—
<i>Rhynchoglossum azureum</i> (Schltdl.)			AJ490895	AJ492282	—
<i>R. notonianum</i> (Wall.) B. L. Burt			AJ490896	AJ492283	—
<i>R. obliquum</i> Blume			AJ490897	AJ492284	—
<i>Stauranthera grandiflora</i> Benth.			AJ490900	AJ492287	—
<i>Whytockia sasakii</i> (Hayata) B. L. Burt			AJ490901	AJ492288	—
<i>W. tsiangiana</i> (Hand. -Mazz) A. Weber			AJ490902	AJ492289	—

* Sequences are newly submitted to GenBank. Voucher information of sequences obtained from GenBank is not provided here. Dashed (—) indicate that no sequence was obtained